

281-Pos**Characterization of Prestin Oligomerization and Diffusion At the Single Molecule Level****Ramsey I. Kamar**¹, Laurent Cognet², Robert M. Raphael¹.¹Rice University, Houston, TX, USA, ²Centre de Physique Moléculaire Optique et Hertzienne, Université Bordeaux, Talence, France.

Prestin is the putative motor protein that drives outer hair cell electromotility. Several groups have shown evidence of prestin-prestin interactions and prestin oligomerization using biochemical techniques and optical imaging. Furthermore, studies have demonstrated that alterations of cholesterol in the cell membrane, which modify prestin function, affect membrane fluidity and prestin mobility. However, the role prestin-prestin interactions or oligomerization play in electromotility and the molecular motifs that mediate these interactions are unknown. It is also unknown whether the effects of cholesterol modifications on prestin function are due to changes in prestin mobility or changes in prestin interactions. Ensemble optical measurements of prestin self-association cannot provide the molecular level details of prestin-prestin interactions, nor can they distinguish the oligomeric states the interactions produce. Likewise fluorescence recovery after photobleaching cannot elucidate non-Brownian modes of diffusion which could indicate interactions with the cytoskeleton. We have thus developed our ability to detect individual prestin-citrine molecules using single molecule fluorescence (SMF) microscopy. We have applied SMF to measure the distribution of intensities emitted by individual prestin clusters in the HEK cell membrane. The distribution displays peaks spaced at multiples of the unitary intensity which one would expect for a distribution of non-interacting fluorescent emitters. We have resolved the stoichiometries up to tetramers, however the data makes clear that higher populations also exist. We have also explored the effect of membrane cholesterol depletion on the oligomerization of prestin using SMF microscopy to assess whether this treatment dissociates prestin oligomers or simply removes a bulk population of intact oligomers from microdomains. We will further utilize total internal reflection fluorescence microscopy and site-directed labeling to measure prestin diffusion at the single molecule level and explore the effects of cholesterol depletion and cytoskeletal inhibitors.

282-Pos**Molecular Dynamics Simulations of the Rotary Motor F₀ Under External Electric Fields Across the Membrane****Yang-Shan Lin**¹, Jung-Hsin Lin², Chien-Cheng Chang¹.¹National Taiwan University, Taipei, Taiwan, ²Academia Sinica, Taipei, Taiwan.

The membrane-bound component F₀, which is one major component of the F₀F₁-ATP synthase, works as a rotary motor and plays the central role of driving the F₁ component to synthesize ATP. We have conducted MD simulations of a b₂-free F₀ in the POPC lipid bilayer for tens of nanoseconds with two different protonation states of the key residue cAsp61 in the absence of electric fields and under electric fields of ± 0.03 V/nm across this membrane. Principal component analysis revealed that the motions of F₀ are clustered by the protonation state of Asp61. Analysis of the residue-pair Pearson correlation coefficient showed that electric fields reduced the correlated motion between the *a*-*c* subunits. When all Asp61 were protonated, the Ala24 and Ile28 of the N-terminal helix rotated clockwise about its axis by 30°. This rotation induced by electrostatic interaction between N-terminal helix and c₁₂ unit may lead the conformational changes in the C-terminal helix which are important for the rotation of *c* ring. Hydrogen bonds networks analysis to the residues on three proton pathways is applied to see whether the hydrogen bonds formed between them. The intrinsic pK_a values of Asp61, Lys203 and Glu219 are supported by the experimental data. The deuterium order parameter (S_{CD}) profile calculated by averaging all the lipids was not very different from that of the pure bilayer, which agrees with recent ²H solid-state NMR experiments (Kobayahi *et al*, Biophys. J., 94, 4339, 2008). However, by delineating the lipid properties according to their vicinity to F₀, we found S_{CD} and lateral diffusion of lipids followed a shell-dependent behavior. These findings may not only be useful to understand the dynamics of F₀, but shed some light to rationalize its extraordinary energy conversion efficiency.

283-Pos**Mechanism of Targeting the a Kinase Anchoring Protein AKAP18δ To the Membrane****Andreas Horner**¹, Frank Goetz², Enno Klusmann², Peter Pohl¹.¹Universität Linz, Linz, Austria, ²Leibniz-Institut für Molekulare Pharmakologie, Leibniz, Germany.

Many proteins bind phospholipids too weakly to direct membrane association on their own. Localization studies nevertheless, reveal membrane anchoring. Membrane anchoring is furthermore considered to be crucial for their function.

AKAP18δ, for example, is part of the signalling cascade which regulates the plasma membrane abundance of the water channel aquaporin-2. The cascade requires both proteins to colocalize in intracellular membranes. In contrast, membrane affinity appears to be rather low as suggested by high sequence homology to the preferentially cytoplasmic AKAP18γ, and the lack of palmitoylation or myristoylation sites which are believed to tailor the homologous proteins AKAP18α and AKAP18β to the membrane. Coincidence detection of a putative binding domain with large net positive charge to negatively charged lipids and specific recognition of a membrane anchored protein (e.g. phosphodiesterase PDE4D) may explain specific membrane targeting of AKAP18δ. Oligomerization of AKAP18δ would also result in an increased membrane affinity by providing several binding sites. To distinguish between both hypotheses, we monitored binding of purified wild type AKAP18δ and AKAP18δ fragments to planar lipid bilayers using fluorescence correlation spectroscopy. Protein binding to both charged and uncharged membranes did not require accessory proteins. Analysis of membrane diffusion constant revealed the existence of oligomers, confirming thereby the second hypothesis.

284-Pos**The Electrostatics of VDAC: Implications in Selectivity and Gating****Om P. Choudhary**¹, Rachna Ujwal², William Kowallis¹, Rob Coalson¹, Jeff Abramson², Michael Grabe¹.¹University of Pittsburgh, Pittsburgh, PA, USA, ²University of California, Los Angeles, Los Angeles, CA, USA.

Voltage-dependent anion channels (VDAC) are transmembrane proteins found in high abundance in the outer mitochondrial membrane of saccharomyces cerevisiae and all higher eukaryotes. VDAC is gated, or opened and closed, by changes in voltage across the membrane as well as pH, and it is thought to mediate the transfer of metabolites such as ATP, ADP, and NADH between the cytoplasm and intermembrane space. Apoptotic regulating proteins interact directly with VDAC to modulate the mitochondrial membrane potential and control the release of cytochrome c during apoptosis. Additionally, altered VDAC permeability has been associated with cancer and cardiovascular disease. Recently the x-ray crystal structure of VDAC from mouse (mVDAC1) was solved at 2.3 Å resolution making it possible to study the molecular workings of this channel in unprecedented detail. Our lab is using computational methods to explore the biophysical properties of the channel. I will discuss the results from continuum electrostatics calculations that show that the channel is selective for anions, which suggests that the x-ray structure is in the open state. This claim is buttressed by Poisson-Nernst-Planck (PNP) calculations that predict a high single channel conductance indicative of the open state. Furthermore, we performed in silico mutagenesis on residues shown to be involved in selectivity, and the changes in the calculated free energy profiles are consistent with experimental changes in selectivity. I will end by discussing how we used membrane potential calculations to rule out a proposed gating mechanism of the channel.

285-Pos**Mapping the SecA-SecY Interaction Interface Using in Vivo Photocross-linking****Sanchaita Das.**

Wesleyan University, Middletown, CT, USA.

In *Escherichia coli* (*E. coli*) the major secretory pathway for proteins is the "general secretion pathway" or "Sec-dependent pathway". Its major components include the SecA ATPase, the SecB chaperone, and SecYEG: the channel-forming complex. Once bound to SecYEG, the preprotein substrate, and ATP, SecA undergoes an ATP-driven conformational cycle that drives the step-wise translocation of proteins. While cytosolic SecA specifically recognizes and binds SecYEG, the specific residues by which these two proteins interact are not known. A study from Mori and Ito (2006) analyzed SecY-SecA interaction by using an in vivo site-directed cross-linking technique developed by Schultz and co-workers. In this experimental system, an amber suppressor tRNA and a tyrosyl-tRNA synthetase from *Methanococcus jannaschii* are genetically engineered to allow for the charging of the tRNA with *p*-benzoyl-phenylalanine (pBpa), a photo-reactive phenylalanine derivative. They showed that pBpa residues introduced into the second, fourth, fifth, and sixth cytoplasmic domains of SecY could be crosslinked to SecA.

We have chosen a similar approach to Mori and Ito, but we have designed sites of in vivo pBpa incorporation into SecA based on an in vivo SecA membrane topology study performed by Jilaveanu *et al.* (2006). The amber positions in SecA were chosen based on residues of SecA that were accessible to small molecule labeling from the exterior side of the membrane-indicating proximity to the SecYEG channel. Our results demarcate novel sites of SecA interaction with SecY. Our data provide in vivo support for the biological relevance of the recent SecA-SecYEG X-ray structure (Zimmer *et al.*, 2008), but they also